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Candidate proto-oncogenes and tumor suppressor genes were previously isolated in a gene expression study involving breast cancer. The present research focus on the characterization of genetic alterations associated with presenilin-2 (PS-2), a gene identified in the gene expression analysis. PS-2 has been implicated in the pathogenesis of Alzheimer's disease, but functional studies indicate that the gene plays a role in pathways commonly perturbed in cancer, hence suggesting that PS-2 may be a target for genetic alterations in tumors. PS-2 maps to a chromosomal region for which LOH/deletion in breast tumors has been reported. We found low PS-2 expression in a fraction of breast tumors. In some cases, the reduction in expression can be attributed to deletion at the PS-2 genomic locus. In addition, we identified two sequence alterations in PS-2 which resulted in amino acid substitutions. Both of the alterations were observed to affect the physiological activity of PS-2, but appear not to be contributing to the Alzheimer's phenotype. The alterations may indeed contribute to mammary tumorigenesis, and we are in the process of further exploring this possibility.

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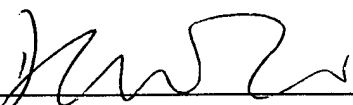

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INTRODUCTION:

The main objective of this research focus on characterizing putative proto-oncogenes and tumor suppressor genes which were identified by differential display PCR in an analysis of gene expression in breast cancer. In particular, the work has focused on further elucidating the putative role of *presenilin-2* (PS-2) in mammary tumorigenesis. PS-2 is implicated in the pathogenesis of Alzheimer's disease, but emerging functional studies indicate that the gene also functions in cancer-related pathways. In addition, PS-2 maps to a chromosomal region for which loss of heterozygosity (LOH)/deletion in breast cancer has been observed, suggesting that a loss of PS-2 function may be an important event during breast cancer pathogenesis. In line with that hypothesis, we observed a reduction in PS-2 mRNA level in a number of breast tumors. This reduction in expression can be partially attributed to deletion at PS-2 genomic locus. In addition, mutational analysis of PS-2 in breast tumors has identified two sequence alterations leading to amino acid substitutions. On going work is aimed at determining the significance of these alterations at the functional levels. The overall goal of this research is to clarify the potential involvement of PS-2 in breast cancer pathogenesis. If the role of PS-2 in breast cancer can be confirmed, it will provide further insights into the etiology of the disease, and ultimately leading to advances in detection, diagnosis, and treatment of breast cancer.

RESEARCH SUMMARY:

PS-2 is a member of an emerging family of proteins with multiple transmembrane domains that localized predominantly to the endoplasmic reticulum and Golgi networks. Although PS-2 has been implicated to have a role in the pathogenesis of Alzheimer's disease, both genetic and clinical studies have indicated that its role in this disease is limited. Functional studies have shown that PS-2 is involved in cellular pathways which are frequently perturbed during tumorigenesis, suggesting that it may also be a target for genetic alterations. In fact, PS-2 maps to 1q41-42, a chromosomal region for which cytogenetic abnormality in breast cancer has been reported. These alterations may compromise PS-2 function in the cell, and thereby disrupting its role in apoptosis, cell proliferation, Notch signaling and wnt signaling. The focus of this work are to identify PS-2 associated genetic alterations in breast cancer, and to determine how these alterations translate to a cellular condition which contribute to tumorigenesis.

1. We have developed a quantitative RT-PCR assay to measure the level of PS-2 mRNA in tumor specimens. The assay is consisted of multiplex PCR reactions in which relative PS-2 expression in any breast tumor sample can be reliably assessed in comparison to a commonly used house-keeping gene. For each tumor specimen, the PCR reactions were performed over a number of different amplification cycles to ensure that quantification is within the logarithmic phase of PCR kinetics. Using this assay, the expression of PS-2 in a cohort of breast tumors was analyzed. Similar to many other cancer genes, a wide range of PS-2 expression in the tumors was observed. However, given reports that PS-2 has proapoptotic activity and is involved in regulating cell cycle progression, we speculated that low level of PS-2 expression might compromise the role of PS-2 in these cellular functions. Indeed, it has been shown that antisense inhibition of PS-2 expression leads to a loss of response to apoptotic stimuli.

2. To further explore the results in point 1, we developed a quantitative DNA PCR to measure PS-2 DNA level in breast tumors for which expression data are also available. The majority of breast tumors displayed a diploid PS-2 DNA content. However, in some cases with low PS-2 expression we also observed a reduced level of PS-2 DNA, indicating that reduced PS-2 expression during mammary tumorigenesis can in some cases be accounted for by deletion at the PS-2 genomic locus. This result further substantiate the suspicion that low PS-2 expression may represent an abnormal condition, but one that supports tumorigenesis. There were also tumors that displayed low PS-2 mRNA level despite a normal level of PS-2 DNA. Clearly, additional mechanisms are involved in deregulating PS-2 expression during breast cancer pathogenesis. It will be important to determine the genetic elements and protein factors involved in regulating PS-2 expression as these may also be subjected to genetic alterations in breast cancer.

3. Using a combination of single strand conformation polymorphism (SSCP) and DNA sequencing, two different sequence alterations in PS-2 have been detected in breast tumors. Both of these alterations lead to amino acid substitutions that are distinct from the two known PS-2 mutations associated with Alzheimer's disease. These cancer-associated alterations occur in proximity of one another, suggesting that they may be

targeting a functionally important domain in PS-2. Unlike the Alzheimer's disease associated mutations, both of the alterations detected in breast tumors did not lead to an increase in secretion of A β (42), the major component in the neuropathic plaques found in brains of patients with Alzheimer's disease. But in using the *C. elegans* model to assess effect of these alterations, we have found that both do compromise the physiological function of PS-2. These results indicate that although the alterations do affect the integrity of the PS-2 protein, they do not appear to be contributing the Alzheimer's phenotype, but perhaps are playing a role in the pathogenesis of cancer. At the present time, we are in the process of assessing the effect of these alterations in cells derived from PS-2 knock-out mice at the level of apoptosis and cell proliferation. For this purpose we have developed a retroviral vector mediate system for ectopic expression of the different PS-2 variant in PS-2 $-/-$ mouse embryonic fibroblasts (MEFs).

A. Research Accomplishments:

- Detection of breast tumors with low expression of PS-2.
- Some tumor with low PS-2 can be attributed to deletion at the PS-2 genomic locus.
- Results also suggest the involvement of additional mechanisms of deregulating PS-2 expression, such as alterations in promoter region or relevant transcriptional regulators.
- Detection of two functional amino acid alterations in PS-2.

B. Reportable Outcomes:

1. Susan J. Done, Minh D. To, and Irene L. Andrulis. Overexpression of LAF-4, a Putative Proto-oncogene in Breast Cancer. Poster presentation at the 1999 Reasons for Hope Breast Cancer Research Conference. Toronto, Ontario, Canada. June 10-12, 1999. (Abstract enclosed).
2. Minh D. To. Potential Involvement of the Alzheimer's disease-associated Presenilin-2 Gene in Tumorigenesis. Podium presentation at the Department of Molecular and Medical Genetics Seminar Series. University of Toronto, Toronto, Ontario, Canada, April 25, 2000.
3. Minh D. To, Timothy G. Doyle, Robert Gryfe, Steve Gallinger, Mark Redston, Peter St. George Hyslop, and Irene L. Andrulis. Potential Involvement of the Alzheimer's disease-associated Presenilin-2 Gene in Tumorigenesis. Podium and Poster presentations at the 2000 Department of Defense Era of Hope Breast Cancer Meeting. Atlanta, Georgia, USA, June 8-12, 2000. (Abstract enclosed).

Appendix

POTENTIAL INVOLVEMENT OF THE ALZHEIMER'S DISEASE-ASSOCIATED PRESENILIN-2 GENE IN TUMORIGENESIS

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We initially isolated presenilin 2 (PS-2) in an analysis of gene expression in breast tumors using differential display. Although PS-2 is one of the familial Alzheimer's disease (FAD) causative genes, several studies suggested that it may also have a role in cancer-related pathways. In particular, the presenilins gene family has been shown to be involved in lin-12/Notch signaling, cell survival/cell death, and regulation of β -catenin. In addition, we mapped PS-2 to a chromosomal region targeted by LOH/deletion, suggesting that its loss may be involved in tumorigenic progression. The aim of this study is to further explore the potential role of PS-2 in tumorigenesis.

Using quantitative RT-PCR we observed low PS-2 mRNA levels in a number of breast tumors. Furthermore, we found the level of PS-2 mRNA to be induced with increasing cell density, suggesting that PS-2 may have a role in cell cycle regulation or contact inhibition. Mutational analysis of PS-2 in breast tumors identified two different sequence alterations which are distinct from the two known FAD-associated mutations. Both alterations result in amino acid substitutions. These amino acid substitutions occur in close proximity of each other, suggesting that they may be targeting a functionally important domain within the PS-2 protein. These alterations are not specific to breast tumors as we have also detected the alterations in colorectal tumors. Analysis of corresponding normal tissues for the colon cancer cases with the alterations indicated that the alterations are constitutive. However, a similar conclusion cannot be made for the breast cancer cases at this time.

We have also performed a number of functional assays to assess the effect of the detected alterations. In contrast to the FAD-associated mutations, the detected alterations did not appear to affect the processing of the amyloid precursor protein, as determined by quantitative measurements of levels of different A β polypeptides. In contrast, in taking advantage of the *C. elegans* model, we found that both detected alterations did compromise the function of PS-2 protein, as determined by measuring the efficiency at rescuing an egg-laying-defect in worms deficient for endogenous presenilin. Altogether, these results suggest that the detected cancer-associated alterations can compromise the function of PS-2 protein and are doing so in a manner distinct from the FAD-associated mutations.